EFFECTS OF ETHANOL ON THE HEPATIC DNA IN MATERNO-FETAL COMPLEX AND ON THE SERUM PROTEINS IN PREGNANT FEMALE RATS

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Abstract
Experimental investigations on the action of ethanol were performed on pregnant female rats and their fetuses. The Wistar strain pregnant rats were included into an experimental (E) and a control (C) group. Animals of experimental group received ethanol and thecontrol group consumed tap water. At the end of the experiment blood samples and liver samples were taken for analysis. In the present study there were pursued values of some biochemical parameters, i.e. maternal and fetal hepatic DNA concentration, maternal serumproteins and electrophoretic fractions (albumins as well as α-, β- and γ-globulins). The obtained data revealed a statistically non-signifiant increase of hepatic DNA both in mothers and fetuses of experimental group. Serumproteins concentration in the pregnant rats showed a significant increase. Regarding the electrophoretic fractions, a decrease of albumins and increase of globulins were observed . As to globulin subfractions a hyper-α-globulinemia, hypo-β- and hypo-γ-globulinemia were revealed.

Key words: ethanol, pregnant rats, biochemical parameters

Introduction
The harmful action of ethanol on mammalian conception products is manifested by morphological and biochemical disorders during prenatal development (embryonic and fetal periods) and also on postnatal development (Kissin, 1971; Sokol, 2003). Clinical observations and experimental research on laboratory animals have led to the circumscription of the concept of fetal alcohol syndrome (FAS), also called alcoholic embryopathy or alcoholic fetopathy (Henderson et al., 1979; Gârban, 1993; Scott, 2014). Our investigations pursued the changes induced by ethanol on the maternal and fetal hepatic DNA as well as on the maternal serum proteins of rats.

Experimental
General design. Investigations were performed on pregnant female rats (Wistar strain) with an average weight of 180-200 g. Rats were included in two groups: one experimental (E) – animals receiving 20% v/v ethanol ad libitum in drinking water and one control (C) - animals consuming only drinking water. Each group comprised 8 animals and had the same feeding and environmental conditions. On day 20th of pregnancy the animals were euthanasied by overdosage of an anesthetic agent. By punction of the vena cava caudalis blood samples were

collected from the pregnant rats and afterwards a hepatic sample was excized for analysis. Liver samples were taken also from fetuses after fetal laparatomy in order to determine the hepatic DNA concentration. Requirements for the protection of animals used in scientific or other experiments were respected (Council Directive 86/609/EEC).

Biochemical investigations. Maternal and fetal hepatic DNA were determined by Spirin`s method (1958) adapted to an UV Vis spectrophotometer (C.Zeiss-Jena). Maternal serum proteins were determined spectrophotometrically based on a reaction with copper sulfate
(λ 550 nm), while the electrophoretic fractions by using paper electophoresis (Kaplan and Pesce, 2010) .

Statistical evaluation. All the obtained experimental data were statistically processed, mean values (X) and standard deviations (SD) were calculated. For this purpose the ANOVA (Analysis of Variance) test was used.

Results and discussions

Experimental data revealed the effects of ethanol - considered as a xenobiotic - on the hepatic DNA biosynthesis and indirectly the disturbance of proteins biosynthesis (Mandal et al., 2017; Gârban, 2018). These aspects give an image on the subcellular mechanisms disturbing the morphogenesis processes triggered by chronic alcohol intake.
Understanding the effects induced by ethanol and its metabolites involves the knowledge of the mechanism of ethanol biodegradation (fig.1). It occurs in two steps: initially ethanol is

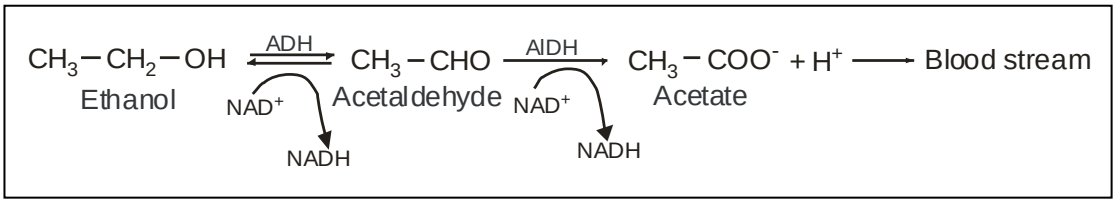

Fig.1. Ethanol biodegradation - general mechanism

transformed to acetaldehyde in cytosol in the presence of the enzyme alcohol dehydrogenase (ADH), afterwards acetaldehyde is converted into acetate in mithocondria under the action of the enzyme aldehyde dehydrogenase (AlDH). The first step of ethanol oxidation, i.e. acetaldehyde formation, can occur in two ways: by the Peroxisomal Ethanol Oxidizing System (PEOS) in which the peroxisomal catalase is involved and by Microsomal Ethanol Oxidizing System (MEOS) in which the CYP2E1 enyzme is acting.

Our experimental data regarding maternal hepatic DNA (Table 1) reveal an increase of the DNA concentration in group E, under the action of ethanol.

Table 1. Concentration of maternal hepatic DNA

Group	**Number of gestant females**	**Hepatic DNA (µg/mg tissue) X ± DS**	**ΔX X_C - X_E**	**Concentration range**
C	8	2.95 ± 0.20	-	2.69 – 3.16
E	8	3.01 ± 0.23	+ 0.06	2.73 – 3.19

In literature studies regarding the action of ethanol on fetal and maternal DNA gained more and more extention. Thus, Dreosti et al. (1981) observed the decrease of DNA in rats brain under the action of ethanol. Investigating the DNA content in various organs: brain, liver, heart, Henderson et al. (1978) decelated also, decreases, mentioning the quantitative depresion on the whole organ and augmentation in the determination in μg/mg hepatic tissue (dosage, possible by lumbar tissue sampling).

Investigations on fetal hepatic DNA – data presented in Table 2 – reveal modifications, i.e. increase (non significant from statistical point of view).

Table 2. Concentration of fetal hepatic DNA

Group	**No of gestant females**	**No of conceptuses**	**No of living fetuses**	**Hepatic DNA (μg/mg tissue) X ± DS**	**ΔX $X_C - X_E$**
C	8	73	72	3.08 ± 0.38	-
E	8	71	65	3.11 ± 0.46	+ 0.03

Table 3. Serumproteins and electrophoretic fractions in pregnant rats

Specification	**Unit**	**Group C**		**Group E**		**ΔX $X_C - X_E$**
		No. anim.	**X_C ± DS**	**No. anim.**	**X_C ± DS**	
Proteins *	g%	8	5.47 ± 0.42	8	5.64 ± 0.59	+ 0.17
Albumins	%	8	56.30 ± 6.12	8	55.20 ± 8.32	- 1.10
Globulins – total			43.70 ± 6.12		44.80 ± 8.32	+ 1.10
α-globulins**			22.70 ± 4.26		25.50 ± 5.63	+ 2.80
β-globulins**			15.20 ± 3.08		14.20 ± 3.96	- 1.00
γ-globulins			5.80 ± 1.60		5.10 ± 1.82	- 0.70

*0.95 < p < 0.99 ** 0.90 < p < 0.95

Results obtained for group C are between characteristic range of species (He et al., 2017). In Group E one can observe the increase of the serum proteins concentration, decrease of albumin fraction and augmentation of globulin fraction, as well as modifications of the globulin subfractions.

Albumins and globulin fractions are involved in the material metabolism. Thus, albumins take part in trophic processes and circulation of diverse inorganic compounds (anions, cations) and small molecule organic compounds. Globulins take also part in trophic and immune processes.

Conclusions

1. Chronic alcohol consumption in case of pregnant rats showed a statistically non-significant increase of their hepatic DNA concentration.
2. Fetuses of the experimental animals revealed also a non-significant increase of the hepatic DNA concentration.
3. A significant increase of serum proteins in pregnant female rats from experimental group was found.

4. Electrophoretic fractions in group E evidenced a decrease of albumins and increase of total globulins. As to globulin subfractions a hyper-α-globulinemia and hypo-β- and hypo-γ-globulinemia were revealed.

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